

REMARKS

Claims 183-318 and 325-400 were previously presented in Applicants' August 20, 1999 Fourth Supplemental Amendment. No changes to the claims have been requested in this Communication. Thus, the same claims continue to be presented for further examination in this application.

In addition to Applicants' May 18, 1999 Third Supplemental Amendment, this Communication also follows the personal interview held on October 26, 1999 in the office of Examiner Ardin H. Marschel, Ph.D., Group Art Unit 1634, and attended by Applicants' undersigned attorney. It is believed that the interview engendered a helpful discussion of the issues in this application. Applicants and their attorney sincerely appreciate the opportunity to clarify the issues that was afforded them by Examiner Marschel at last week's interview.

In the Examiner Interview Summary Record, it was indicated that:

We discussed whether there is written basis for different strands immobilized on the substrate of the array claim 325 as well as specific [dependent] claims therein. Mr. Fedus intends to file a communication shortly regarding full support for [claim] 325. . .

As currently worded, claim 325 recites:

An array of substrate surfaces, each substrate surface comprising at least one double-stranded nucleic acid fixed or immobilized thereto, wherein at least one strand comprises one or more chemical labels which comprise a signaling entity or entities which are quantifiable or detectable, and wherein at least one nucleic acid strand or a sequence therefrom in one of said substrate surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another substrate surface.

The Examiner's query concerned the language "wherein at least one nucleic acid strand or a sequence therefrom in one of said substrate surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another substrate surface." In response, Applicants offer the following remarks.

The language in claim 325 with respect to different nucleic acid strands being fixed or immobilized to the array's substrate surfaces is wholly supported by the specification. In the specification, for example, on page 16, first full paragraph, Applicants disclose:

Such treated glass could then be employed in the practice of the method of the invention. For example, glass plates provided with an array of depressions or wells would have samples of the various denatured analytes deposited therein, the single-stranded analytes being fixed to the surfaces of the wells. Thereupon, polynucleotide probes provided with a chemical label may be deposited in each of the wells for hybridization to any complementary single-stranded analyte therein. After washing to remove any non-hybridized probe, the presence of any hybrid probe-analyte is detectable according to the method of the present invention. One detection technique as described herein involves the addition of an enzyme-linked antibody or other suitable bridging entity of the label for attachment of the probe. Subsequently a suitable substrate is added to elicit the soluble signal, e.g., a color change or chemical reaction, which is then measured colorimetrically or photometrically. (emphasis added)

It is quite clear from the above-quoted paragraph that Applicants not only envisioned but that they fully described in their original specification the placement of different nucleic acid sequences or strands on the substrate surfaces in the claimed array at hand. In the emphasized statements quoted above, Applicants disclose that the " . . . array . . . would have samples of the various denatured analytes deposited therein, the single-stranded analytes being fixed to the surfaces . . ." The dispositive word on this matter appears in the last-quoted phrase in the form of the term "various." According to several well-known dictionaries, the adjective "various" uniformly refers to differences among items or things, in this case, the double-stranded nucleic acid strands. Merely for illustration purposes only, resort may be made to any of the following definitions taken from several well-known dictionaries:

**various** *adj. Abbr. var.* 1. a. Of diverse kinds. b. Unlike, different. 2. More than one; numerous; several. 3. Many-sided; varying; versatile. 4. Having a variegated nature or appearance. 5. Being one of a class or group; individual and separate: *The various reports all agreed.* 6. *Archaic.* Changeable; variable. . . —*var'i\*ous\*ly* *adv.* —*var'i\*ous\*ness* *n.*

**Usage:** *Various* sometimes appears as a plural collective pronoun followed by *of* rather than by a noun, but the usage is widely condemned: *He spoke to various of the members* (or *spoke to various*

of them). The preceding example is termed unacceptable by 91 per cent of the Usage Panel. *Various* has its proper function as an adjective in *He spoke to various members.*

The American Heritage Dictionary Of The English Language, Houghton Mifflin Company, Boston, William Morris, *Editor*, 1978, page 1417, copy attached as Exhibit A.

**various** *adj.* 1. differing one from another, or of different kinds, as two or more things. 2. divers, several, or many: *in various parts of the world*. 3. exhibiting or marked by variety or diversity. 4. differing in different parts, or presenting differing aspects. . .

—**Syn.** 1. VARIOUS, DIFFERENT, DISTINCT, DIVERSE refers to things which are sufficiently unlike to be perceptibly of more than one kind. VARIOUS implies that there are several kinds of the same general thing: *various types of seaweed*. DIFFERENT is applied either to a single thing differing in identity or character from another, or to two or more things differing thus from one another: *two different stories concerning an event*. DISTINCT implies want of connection between things, which, however, may possibly be unlike or similar: *two distinct accounts which coincide*. DIVERSE commonly implies a number or assortment of things or parts differing one from another: *three completely diverse proposals for preventing inflation*.

—**Ant.** 1. identical, same, similar, uniform.

The American College Dictionary, Random House, Inc., New York and Toronto, 1963, page 1345, copy attached as Exhibit B.

**various** *adj.* . . . 1 *archaic*: VARIABLE, INCONSTANT 2 VARICOLORED <birds of ~ plumage> 3 a : of differing kinds : MULTIFARIOUS b : dissimilar in nature or form : unlike <animals as ~ as the jaguar and the sloth> 4 : having a number of different aspects or characteristics <a ~ place><a ~ talent> 5 : of an indefinite number greater than one <stop at ~ towns> 6 : INDIVIDUAL, SEPARATE <rate increases granted in the ~ states> *syn* see DIFFERENT—**variousness** *n*

Webster's Ninth New Collegiate Dictionary, Merriam-Webster, Inc., Springfield, MA, 1988, page 1305, copy attached as Exhibit C.

**various**, *a.* 1. differing one from another; of several kinds. 2. several; many; as, *various sections of the country*. 3. many-sided; versatile. 4. characterized by variety; varied in nature or appearance. 5. changeable. [Rare.]

Webster's New Universal Unabridged Dictionary, 2nd edition, New World Dictionaries/Simon and Schuster, New York, Jean L. McKechnie, *editor*, 1979, page 2022, copy attached as Exhibit D.

Applicants are also mindful of their originally filed abstract which states:

Polynucleotide sequences in a sample of biological or nonbiological material are detected by a method involving fixing of the sequences on a solid support and forming an entity between the fixed sequences and chemically-labeled polynucleotide or oligonucleotide probes having a sequence complementary to the fixed sequence for determining the identification and/or presence of the target polynucleotide sequences. The chemical label covalently or non-covalently attached to the probe comprises a signalling moiety capable of generating a soluble signal detectable by spectrophotometric assay techniques. (emphasis added)

In light of the above-cited portions of the originally filed specification, Applicants' foregoing remarks and the attached exhibits (A-D), it is respectfully submitted that the language in claim 325 with respect to "at least one nucleic acid strand or sequence . . . in one of said substrate surfaces [being] different from at least one nucleic acid strand or a sequence therefrom in another substrate surface" is fully supported and described as required under the law. The specification quoted above shows that Applicants had possession of their claimed array subject matter in claim 325 when their application was first filed. Thus, the subject matter of claim 325 meets the statutory test for written description.

Also discussed briefly during the October 26, 1999 interview was the matter of the surface treatment claims 326-333 which depend from Applicants' array claim 325. These dependent claims include the following:

<u>Claim No.</u>	<u>Description/Recitation</u>
326	wherein each of said substrate surfaces has been treated with a surface treatment agent
327	wherein said surface treatment agent comprises an amine or amide compound
328	wherein said amine compound is selected from the group consisting of duodecadiamine (DDA), polylysine (PPL), aminopropyltriethoxysilane and a combination . . .
329	wherein said amide compound comprises formamide

- 330 wherein said surface treatment agent comprises a dispersive compound
- 331 wherein said dispersive compound comprises ammonium acetate
- 332 wherein said surface treatment agent comprises an epoxy compound
- 333 wherein said surface treatment agent is selected from . . . an amine compound and an epoxy compound

With respect to the support for the above dependent claims in the specification, Applicants wish to point out that the first full paragraph on page 16, quoted and discussed *supra*, begins significantly with a reference to "treated glass":

Such treated glass could then be employed in the practice of the method of the invention. For example, glass plates provided with an array of depressions or wells would have samples of the various denatured analytes deposited therein . . . (emphasis added)

The treatment to the support referred to above follows, of course, the opening of the DETAILED DESCRIPTION section and Example 1 on page 15 which continues into the next page (page 16), including the above-quoted portion discussion of Applicants' arrays. There, in the DETAILED DESCRIPTION and Example 1, Applicants disclose:

#### DETAILED DESCRIPTION

The following examples are illustrative of preferred embodiments of the method of the present invention. Specifically referred to therein are methods for fixing the analyte to a non-porous solid support, as well as illustrations of the use of soluble signals in polynucleotide probes as discussed above.

#### Example 1

For purposes of the present invention, an analyte is immobilized on a solid support, preferably a non-porous translucent or transparent support. To effect easy fixing of a denatured single-stranded DNA sequence to a glass support, [one] exemplary "fixing" procedure may involve pretreating the glass by heating or boiling for a sufficient period of time in the presence of dilute aqueous nitric acid. Approximately forty-five minutes in 5% dilute acid should be adequate to leach boron residues from a borosilicate glass surface. The treated

glass is then washed or rinsed, preferably with distilled water, and dried at a temperature of about 115°C, for about 24 hours. A 10 percent solution of gamma-aminopropyltriethoxysilane, which may be prepared by dissolving the above-identified silane in distilled water followed by addition of 6N hydrochloric acid to a pH of about 3.45, will then be applied to the glass surface. The glass surface is then incubated in contact with the above-identified silane solution for about 2-3 hours at a temperature of about 45°C. The glass surface is then washed with an equal volume of water and dried overnight at a temperature of about 100°C. The resulting treated glass surface will now have available alkylamine thereon suitable for immobilizing or fixing any negatively charged polyelectrolytes applied thereto. [See Weetal, H. H. and Filbert, A. M., "Porous Glass for Affinity Chromatography Applications", Methods in Enzymology, Vol. XXXIV, Affinity Techniques Enzyme Purification: Part B. pp. 59-72, W. B. Jakoby and M. Wilchek, eds.] (emphasis added)

Later in their disclosure, Applicants disclose other surface treatments, including those described in Example 5 on pages 21 and 22. In Example 5, Applicants disclose:

#### Example 5

The advantages of the practices of this invention are also obtainable when the probe is immobilized on a non-porous plastic surface. When a plastic surface is employed, it is sometimes desirable to increase the effectiveness or uniformity of the fixation by pretreating the plastic surface.

Because polystyrene from various batches or sources exhibits different binding capacities, the adherence or fixing of DNA to a polystyrene surface is improved by treating the surface with an amino-substituted hydrophobic polymer or material. Previous experiments demonstrated that addition of duodecadiamine (DDA) to polystyrene resulted in an uniform binding coefficient of polystyrene plates of different batches. Another technique for improving the fixing or uniformity of the plastic surface for fixing DNA involves the treatment of the surface with polylysine (PPL).

In tests involving the fixing of DNA to a plastic surface, biotinylated DNA (b-DNA) was denatured and aliquoted into Dynatech, Immulon 11" removable wells. Samples were allowed to dry onto the plastic surface at 37°C. The amount of bound b-DNA was determined by sequential addition of goat anti-biotin antibody and rabbit anti-goat antibody complexed to the signalling moiety, alkaline phosphatase, followed by development with p-nitrophenyl phosphate in diethanolamine buffer, pH 9.6. Enzymatic activity was monitored at 405 nm utilizing the automatic Dynatech Micro ELISA Scanner. This procedure enables quantification of the amount of bound DNA and therefore the degree of biotinylation. To increase the sensitivity of detection, a fluorogenic substrate such as 4-methylumbelliferyl-phosphate, or its analogues, with companion enzymes, may be used.

In a further example of the method, denatured adenovirus 2 DNA, the analyte, was bound to polystyrene plates, as described above. (emphasis added)

Subsequently in their next two examples, Applicants describe other fixing techniques. In Example 6, they disclose:

#### EXAMPLE 6

In further tests, radioactively-labeled DNA was prepared by nick translation with [<sup>3</sup>H]dATP. The labeled, non-biotinylated denatured DNA[2000 ng to 5 ng] was applied to DNA-coated polystyrene plates. The test samples of plates were not allowed to dry. After incubation at 37°C for periods of 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 18 hours, samples were counted. Binding was maximal after two hours of incubation, however, 50 percent of the originally applied DNA bound regardless of the concentration, thereby indicating that there is an equilibrium between bound and unbound DNA.

In other tests, polystyrene microfilter wells were nitrated using the procedure of Filipsson and Hornby, *Biochem. J.* 120, 215 (1970). The polystyrene wells were immersed for 20 minutes in a mixture of concentrated nitric acid and sulfuric acid [41 percent, v/v] cooled to 0°C. The wells were then washed thoroughly with water and subsequently heated to 70°C in a 6 percent solution of sodium dithionite in 2M potassium hydroxide. After 4 hours, the wells were washed thoroughly with 0.5M hydrochloric acid and distilled water.

To produce 6-aminohexane linked polystyrene, 6-amino-caproic acid-N-hydroxysuccinimide ester•hydrobromide [5 mg thereof dissolved in 0.2M dimethylformamide prepared by reacting 6-aminocaproic acid•hydrobromide with N-hydroxysuccinimide and dicyclohexyl carbodiimide in dimethylformamide and recrystallized from isopropylalcohol] was added to 0.1M sodium borate [0.4ml]. Amino-derivitized polystyrene microfilter wells filled with this solution were allowed to react at room temperature for 4 hours and then washed thoroughly with distilled water. The resulting treated cells absorbed H-labeled DNA from aqueous solution at pH less than 9.5.

An improved capability for fixing or immobilization of DNA to non-porous siliceous solid supports, such as glass and plastic, is also provided by treatment with a coating of an epoxy resin. For example, treatment of glass or polystyrene surfaces with commercially available epoxy glue, such as a solution of epoxy glue in ethanol [1 percent w/v] serves this purpose. These epoxy solutions are applied to the surfaces or wells, and the solvent, ethanol, evaporated thereupon at a temperature of 37°C, thereby providing a polyamine polymeric coating on the treated surface. These surfaces were found to absorb <sup>3</sup>H-labeled DNA from aqueous solution at pH less than 9.5.

(emphasis added)

In the next example, Applicants further disclose:

EXAMPLE 7

Yet another example of the method of the present invention, including fixing the polynucleotide analyte sequence directly to a non-porous solid support, such as a conventional microtiter well, may be performed according to the procedures outlined below.

Conventional microtiter well plates can be pre-rinsed with 1M ammonium acetate (NH<sub>4</sub>OAc), in an amount of 200 $\mu$ l/well. Analyte DNA would be diluted to 10-200 ng/50 $\mu$ l in water or 10 mM Tris-HCl at pH 7.5 and 1mM EDTA(TE). After boiling for 5 minutes and quick cooling in ice water, an equal volume of 2M NH<sub>4</sub>OAc would be added and 50  $\mu$ l of analyte DNA is added per well, giving 5-100 ng of analyte DNA per well. After open plate incubation for 2 hours at 37 °C until the wells are dry, at which point the plates can be sealed, and stored at 4 °C for up to one-two months. Single-stranded analyte DNA is now fixed to the wells.

An alternative method to denature and then fix the analyte DNA to the well is to add 50 $\mu$ l of DNA in TE to wells at a concentration of 10-200ng/50 $\mu$ l. After adding 25 $\mu$ l at 0.9N NaOH and mixing, the plates can be incubated for 10 minutes at room temperature. After adding 25 $\mu$ l of 4M NH<sub>4</sub>OAc, the open plates may be incubated at 37 °C for 4 hours or until dry and the plates sealed and stored at 4 °C until ready to use.

To prepare the plates for hybridization, the wells would be rinsed twice with 0.3m NaCl, 0.03m sodium citrate (2X SSC) (200 $\mu$ l/well) buffer regardless of whether the wells can be rinsed once with 2X SSC/1% Triton X-100 after the two 2X SSC rinses. Plates should be blotted on absorbent paper before beginning each rinse.

To hybridize the fixed analyte with a probe, the following protocol would be followed. . . (emphasis added)

It is quite clear from the above discussion that the specification, particularly in the form of Examples 1 and 5-7 quoted above, fully supports and sufficiently describes the various treatments effected to the substrate surfaces, as set forth in dependent claims 326-333.

Favorable action on all of the pending claims, including Applicants' array claims, is respectfully requested.

\* \* \* \* \*

**SUMMARY AND CONCLUSIONS**

Claims 183-318 and 325-400 continue to be presented for further examination.

No other fee is believed due in connection with this Communication, a three month extension fee having been previously authorized in connection with Applicants' July 21, 1998 Amendment Under 37 C.F.R. §1.115. In the event that any other fee or fees are due, however, either in connection with this Communication or with any of Applicants' previous filings, The Patent and Trademark Office is hereby authorized to charge the amount of any other such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If it would be helpful to expediting the prosecution of this application, the undersigned may be contacted by telephone at 212-583-0100 during the daytime business hours.

Early and favorable action on this application is respectfully sought.

Respectfully submitted



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